

CO₂ 浓度升高和不同氮源对紫茎泽兰生长及光合特性的影响*

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摘要: 大气 CO₂ 浓度升高和植物入侵是全世界面临的两大重要问题。CO₂ 浓度升高促进植物的光合作用, 但在某些植物中, 这种促进作用出现在短期高浓度 CO₂ 下, 而在长期高浓度 CO₂ 处理下消失 (称为 CO₂ 驯化), 被认为源于高浓度 CO₂ 对光呼吸和 NO₃⁻ 同化的抑制。通过比较研究不同形式氮源 (全氮、硝态氮) 和短期 (8 days)、长期 (40 days) CO₂ 浓度升高处理对入侵植物紫茎泽兰生理特征的影响, 结果表明在全氮供应下, 短期和长期 CO₂ 浓度升高均促进了紫茎泽兰的光合; 氨态氮缺失情况下, 长期 CO₂ 浓度升高促进紫茎泽兰的光合, 而短期 CO₂ 浓度升高对紫茎泽兰的光合没有促进作用; 缺 NH₄⁺ 下, 短期高浓度 CO₂ 提高了叶片叶绿素含量, 长期 CO₂ 升高又使其回复到正常 CO₂ 下的较低水平。这些结果表明紫茎泽兰并不会对长期的 CO₂ 升高产生驯化, 即长期 CO₂ 升高会促进紫茎泽兰的光合作用, 而且这一促进作用不受土壤中缺 NH₄⁺ 的影响。鉴于培养介质中缺 NH₄⁺ 会导致一些植物产生 “CO₂ 驯化”, 未来 CO₂ 浓度升高情况下, 在缺 NH₄⁺ 的土壤中, 紫茎泽兰的竞争力可能会更强。

关键词: CO₂ 浓度升高; 紫茎泽兰; 入侵植物; 光合驯化; NO₃⁻ 吸收

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Influence of Elevated CO₂ Concentration and Nitrogen Source on Photosynthetic Traits in the Invasive Species *Eupatorium adenophorum* (Asteraceae)

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Abstract: Increases in the concentration of atmospheric CO₂ and plant invasion are two important problems that face humans worldwide. In some plants, exposure to a short-term elevated concentration of CO₂ (SE [CO₂]) promotes photosynthesis, but the promotion of elevated [CO₂] (E [CO₂]) to photosynthesis might disappear after long-term treatment (so-called “CO₂ acclimation”); this might result from the associated inhibition of nitrate assimilation. The present study investigated the physiological effects of short-term (8 days) and long-term (40 days) exposure to E [CO₂] when these were combined with different forms of inorganic N (full N; nitrate (NO₃⁻)-N) in the invasive species *Eupatorium adenophorum*. Exposure to E [CO₂] increased the biomass of *E. adenophorum*, regardless of the duration of exposure to E [CO₂] and the type of inorganic N that was supplied. E [CO₂] could promote the photosynthesis of *E. adenophorum* seedlings fertilised with non-depleted Hoagland solutions (full N). For plants fertilised

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with NH_4^+ -depleted Hoagland solution (NO_3^- -N), LE $[\text{CO}_2]$ treatment promoted the photosynthesis of *E. adenophorum*, but the promotion of photosynthesis by E $[\text{CO}_2]$ disappeared under SE $[\text{CO}_2]$ conditions. Photosynthetic pigments contents were determined to estimate potential changes in the photosynthetic capacity of *E. adenophorum*. For plants fertilised with non-depleted Hoagland solution, there were no significant differences in chlorophyll among the three $[\text{CO}_2]$ treatments, but the treatment of SE $[\text{CO}_2]$ increased the levels of chlorophyll in leaves. The apparent promotion of biomass accumulation and photosynthesis at LE $[\text{CO}_2]$ without a decrease in chlorophyll indicates that *E. adenophorum* might not acclimate to long-term exposure to E $[\text{CO}_2]$. NH_4^+ depletion did not affect the capacity of LE $[\text{CO}_2]$ to promote the photosynthesis of *E. adenophorum*. Thus, considering some plants fertilised with NO_3^- acclimating to LE $[\text{CO}_2]$, *E. adenophorum* might be more competitive in areas where the soils are relatively poor in NH_4^+ as levels of atmospheric CO_2 continue to rise.

Key words: Elevated CO_2 ; *E. adenophorum*; Invasive plants; Photosynthesis acclimation; NO_3^- assimilation

Atmospheric concentrations of CO_2 ($[\text{CO}_2]$) have increased by more than 0.01% (from 0.027% to 0.0379%) since the beginning of the industrial revolution, and are predicted to rise to 0.073% or even 0.102% by the end of the century (Solomon *et al.*, 2007). Besides being a major greenhouse gas that causes global warming, CO_2 is also the main source of carbon for photosynthesis. Elevated $[\text{CO}_2]$ (E $[\text{CO}_2]$) will have a complicated influence on plant physiology and growth, as well as species distributions (Smith *et al.*, 2013; Kooij and Kok, 1996).

E $[\text{CO}_2]$ affects plant growth and development by changing patterns of photosynthetic carbon assimilation (Reddy *et al.*, 2010), water-use efficiency (Jackson *et al.*, 1994) and flowering time through impacting the rate of leaf production (Song *et al.*, 2009). E $[\text{CO}_2]$ can also delay the senescence of C_3 plants (Curtis *et al.*, 1989). Whereas short-time exposure to E $[\text{CO}_2]$ in controlled experimental conditions increases CO_2 assimilation rates, prolonged exposure often results in “acclimation”, with either no variation or a decrease of photosynthesis; this has been observed in *Arabidopsis*, wheat, tomato, maize and alpine grassland (Bloom *et al.*, 2002; Cousins and Bloom, 2003; Searles and Bloom, 2003; Körner *et al.*, 1997). The availability of N affects this plant response to E $[\text{CO}_2]$. Fertilisation of wheat with nitrate (NO_3^-) was less efficient in stimulating growth under elevated $[\text{CO}_2]$ conditions than fertilisation with ammonium (NH_4^+) (Bloom *et al.*, 2002). Moreover, E $[\text{CO}_2]$ inhibited NO_3^- as-

similation in wheat and *Arabidopsis*; this might play an important role in the decline of photosynthesis and growth of C_3 plants after prolonged exposure to high CO_2 (Bloom *et al.*, 2010). N is one of the mineral elements for which plants have the highest demand (Epstein and Bloom, 2004). Climate change has contributed to increased levels of N deposition in recent decades (IPCC, 2007). Elevated N deposition reduces species diversity (Stevens *et al.*, 2004). Nonetheless, elevated CO_2 can ameliorate the negative effects of N enrichment on species richness (Reich, 2009). In terms of inorganic N in soil, NO_3^- and NH_4^+ are the primary sources for terrestrial plants. The extent to which plants use NO_3^- versus NH_4^+ as N sources varies among species. For instance, regardless of prior N provision, NH_4^+ uptake in seedlings of Scots pine (*Pinus sylvestris*) and European larch (*Larix decidua*) was consistently higher than that of NO_3^- (Bowler and Press, 1996). However, *Vaccinium arboreum* can assimilate NO_3^- efficiently and tolerate an environment where NO_3^- is the predominant form of N (Darnell and Hiss, 2006). This introduces the fundamental question of whether E $[\text{CO}_2]$ could inhibit NO_3^- assimilation in plants that preferred NO_3^- , and thus enable “ CO_2 acclimation”.

Eupatorium adenophorum, a notorious weed that causes major economic losses worldwide, was first introduced into Yunnan Province in Southwest China in the 1940s, and is now spreading into Northern and Eastern China (Sang *et al.*, 2010). The suc-

successful invasion of *E. adenophorum* can be attributed to its remarkable plasticity to adapt to different environments through flexible regulation of its seed germination, photosynthesis, respiration, and capacity to secrete the phytotoxin *o*-coumaric acid to compete with its neighbours (Li and Feng, 2009; Niu *et al.*, 2011; Zheng *et al.*, 2012). Besides this allelopathic strategy to deter competitors, *E. adenophorum* can also alter the micro-environment of ground soil to enhance its suitability for growth; indeed, the soil content of NO₃⁻ was nearly double that of NH₄⁺ in a habitat that had been invaded extensive by *E. adenophorum* (Li *et al.*, 2009). Lei *et al.* (2012) also found that synergistic interactions of E [CO₂] and N deposition might exacerbate invasion by *E. adenophorum*. However, little is known about the effects of different forms of inorganic N on the response of *E. adenophorum* plants to high CO₂.

The rising atmospheric level of the greenhouse gas CO₂ and biological invasions are two major threats to biodiversity worldwide. Accordingly, investigation of the performance of invasive plants under conditions of E [CO₂] is of great importance if plant invasiveness is to be controlled. In the present study, we investigated the effects of long-term (40 days) and short-term (8 days) CO₂ enrichment (0.076%) on the physiological performance of invasive *E. adenophorum* when these were combined with the supply of different forms of N (full N; NO₃⁻-N). Two questions were addressed: (1) In terms of its photosynthesis, does *E. adenophorum* acclimate to E [CO₂] after long-term treatment? (2) What is the influence of different forms of N on the response of *E. adenophorum* to E [CO₂]?

1 Materials and methods

1.1 Plant material

Seeds of *E. adenophorum* were collected from the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan Province, in 2008. All seeds were sterilized with ethanol (70%) for 2 min and sodium hypochlorite

(5%) for 2 min, and then rinsed three times with sterile distilled water. Surface-sterilized seeds were kept at 4 °C for two days before germinating the seeds and growing the seedlings on 1/2 MS (Murashige and Skoog, 1962) medium that contained 0.3% gellan gum (G1910; Sigma-Aldrich) for four weeks. Four-week-old seedlings were grown in 1/4 Hoagland solution for hydroponic culture as described by Tocquinet *et al.* (2003). The conditions in the growth chamber were 22/18 °C, a 12/12 h light/dark cycle, with a relative humidity (RH) of 65% and photosynthetic photon flux density (PPFD) of 120 μmol m⁻²s⁻¹.

1.2 Experiment design

The environmental conditions to which *E. adenophorum* was exposed involved combinations of three CO₂ treatments and two kinds of nitrogen nutrients. Seeds were germinated and grown for 4 weeks. Four-week-old seedlings were subjected to various concentrations of CO₂ and nitrogen nutrients, which were provided in different combinations. All treatments were performed in two closed-top chambers (E-sheng Tech. Co., Beijing, China). The CO₂ was supplied as compressed CO₂ gas, with the CO₂ concentration controlled automatically with a computer-controlled CO₂ supply system (LT/ACR-ePLC, E-Sheng Tech. Co., Beijing, China). The humidity and temperature in the chamber were also controlled by the computer. The concentrations of ambient and elevated CO₂ provided were 0.038% and 0.076% CO₂, respectively. For long-term CO₂ treatments, seedling growth occurred under ambient (0.038%) and elevated (0.076%) CO₂ conditions, indicated by the letter codes LA (long-term ambient) and LE (long-term elevated), respectively. For short-term E [CO₂] treatment, 32-day-old hydroponic seedlings grown with ambient CO₂ concentration were transferred to elevated CO₂ conditions and grown for eight days; this was indicated by the letter code SE (short-term elevated). Treatments with different forms of nitrogen nutrients included Hoagland solution (full N) and NH₄⁺-depleted solution (NO₃⁻-

N). In the solution in which NH_4^+ was depleted, the NH_4^+ was replaced by Na^+ . For example, NH_4NO_3 was replaced by NaNO_3 , at the same molar concentration. Nutrient solution was replaced twice during the growing season. Every treatment combination involved 12 replicates, and each experiment was repeated three times.

1.3 Gas exchange measurements

Gas exchange measurements were carried out *in situ* using a portable gas analysis system, LI-COR 6400 (LI-COR, USA), equipped with a 6 cm^2 LED chamber (LI-6400-02B). Measurements were performed after 5–10 min of stabilization at light-saturating PPFD of $900\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$, ambient humidity (55%–65%) and gaseous flow rate of $300\text{ }\mu\text{mol s}^{-1}$ through the gas exchange chamber. Photosynthetic responses to intercellular CO_2 concentration (C_i) were determined on new fully expanded leaves (the third leaf from the top) of three individuals per treatment. CO_2 concentration was controlled using an LI-6400 CO_2 injector system (LI-6400-01). Light-saturated $A-C_i$ curves started with an ambient CO_2 concentration of 0.038%, followed by decreases to 0.03%, 0.02%, 0.01%, 0.005% and 0, and then increases to 0.005%, 0.01%, 0.015%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08%, 0.1%, 0.12%, 0.14%, 0.16% and 0.2%. Leaves were allowed to equilibrate for at least 3 min at each step before logging data. Water use efficiency (WUE) was estimated as follows: $\text{WUE} = \text{net photosynthesis rates to transpiration rates ratio (Pn/Tr)}$.

1.4 Seedling biomass and photosynthetic pigment content

Seedlings were divided into roots and aerial parts and dried for three days in an oven at $70\text{ }^\circ\text{C}$ before their dry weights were measured. Chlorophyll contents were measured as described by Porra *et al.* (1989). Briefly, after assessment of the photosynthetic rate, half of the leaf was cut and immersed in 8 mL of N, N-dimethylformamide at $4\text{ }^\circ\text{C}$ overnight. The absorbance of the supernatant was detected at 647, 664 and 480 nm. Then, the glass tubes that

contained the leaf were oven-dried for three days at $70\text{ }^\circ\text{C}$ and the sample dry weights were measured. The photosynthetic pigment concentrations were calculated using the following equations: total micrograms of chlorophyll *a* per litre = $[12.00 \times (A_{664})] - [3.11 \times (A_{647})]$; total micrograms of chlorophyll *b* per litre = $[20.78 \times (A_{647})] - [4.88 \times (A_{664})]$; and total micrograms of carotenoid per litre = $\{[1000 \times (A_{480})] - [1.12 \times \text{Ca}] - [34.07 \times \text{Cb}]\} / 245$. Levels of photosynthetic pigments were then determined according to the volume of extraction buffer and the dry weight of the sample.

1.5 Determination of NO_3^- content

The content of inorganic NO_3^- was determined as described by Cataldo *et al.* (1975), with minor modifications. Each frozen leaf sample was ground in the liquid nitrogen. The ground samples were suspended in 1.5 mL of deionised water and the mixture was centrifuged at $18\,000 \times g$ for 15 min. Then, 0.4 mL of salicylic acid (5%) was added to 0.1 mL of supernatant, and the solution was incubated at room temperature for 20 min; subsequently, 9.5 mL of $2\text{ mol} \cdot \text{L}^{-1}$ NaOH was added to raise the pH to above 12. Samples were then cooled to room temperature and absorbance at 410 nm was determined. Similarly, 0.1 mL of standards containing 1–60 μg of NO_3^- -N were mixed, measured as mentioned above and analysed with each set of samples. After the determination of NO_3^- levels, the microcentrifuge tubes with the pelleted sample were oven-dried for three days at $70\text{ }^\circ\text{C}$, and the sample dry weights were measured. Levels of NO_3^- were determined by considering the volume of extraction buffer and the dry weight of the sample.

2 Results and discussion

2.1 Elevated $[\text{CO}_2]$ enhances the growth of *E. adenophorum*

We studied the influence of different nitrogen nutrients on the response of *E. adenophorum* to E $[\text{CO}_2]$. Plants grown in hydroponic solutions where NO_3^- was the sole inorganic N source did not show a

significant phenotypic difference in terms of response to [CO₂] changes compared with the seedlings grown in non-depleted Hoagland solution. Our subsequent studies investigated the influence of NH₄⁺ depletion on the response of *E. adenophorum* to E [CO₂]. Regardless of whether plants were grown in non-depleted Hoagland solutions or NH₄⁺-depleted Hoagland solutions, LE [CO₂] always promoted a substantial increase in *E. adenophorum* biomass. The changes in biomass resulted from changes in the aerial parts of the plants; the root weight did not show a major difference between each treatment (Fig. 1A). Plant height was substantially promoted under LE [CO₂], but not SE [CO₂] (Fig. 1B). The lengths of roots of *E. adenophorum* grown under all treatments had no significant difference (Fig. 1C). The specific leaf area (SLW) was greater in LE [CO₂]-than in either LA [CO₂]- or SE [CO₂]-treated plants. The different N sources tested did not influence the response of SLW to E [CO₂] (Fig. 1D).

There was also no significant difference in ratio of root to shoot dry weight in *E. adenophorum* among all treatments (Fig. 1E). These results might indicate that *E. adenophorum* can use NO₃⁻ as the sole inorganic N source, and that E [CO₂] greatly accelerated the growth of the plants.

2.2 Interactions of elevated CO₂ concentration and nitrogen source on photosynthesis of *E. adenophorum*

LE [CO₂] greatly enhanced the net photosynthesis rates (P_n) of *E. adenophorum*, and the N forms in cultured solutions did not influence this enhancement. However, under SE [CO₂] conditions, this enhancement occurred in seedlings grown in non-depleted Hoagland solution, but not in NH₄⁺-depleted Hoagland solution (Fig. 2A). LE [CO₂] did not influence the stomatal conductance (g_s) of *E. adenophorum* under either of the N treatments tested. NH₄⁺ depletion promoted the closure of stomata in LA-treated plants, which was indicated by

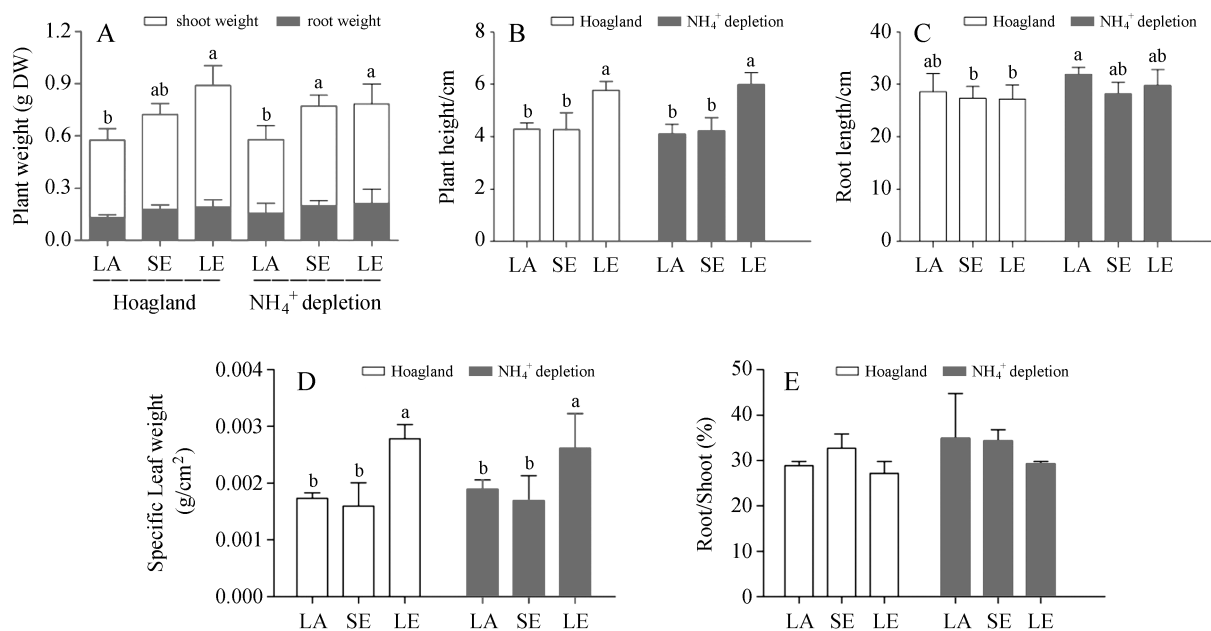


Fig. 1 Differences in growth and morphological characteristics of *E. adenophorum* grown under conditions of long-term ambient CO₂ concentration (LA), short-term elevated CO₂ concentration (SE) and long-term elevated CO₂ concentration (LE) when plants were fertilised using Hoagland solution (Hoagland) and NH₄⁺-depleted solution (NH₄⁺ depletion). A. plant weight; B. plant height; C. root length; D. SLW (specific leaf weight); E. ratio of root to shoot dry weight. Different letters indicate significant differences ($P < 0.05$) among treatments according to one-way ANOVA. Values of plant weight, SLW and the ratio of root to shoot dry weight are means \pm SD of four replicates; values of plant height and root length are means \pm SD of seven replicates; all experiments were repeated three times

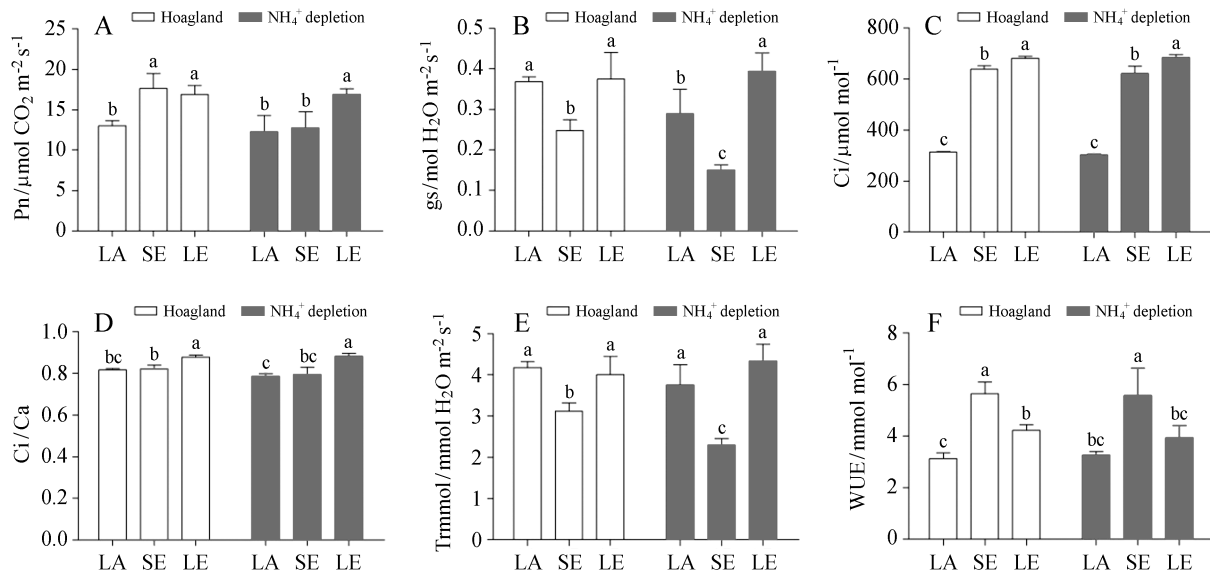


Fig. 2 Differences in gas exchange in *E. adenophorum* grown under conditions of long-term ambient CO_2 concentration (LA), short-term elevated CO_2 concentration (SE) and long-term elevated CO_2 concentration (LE) when fertilised with Hoagland solution (Hoagland) and NH_4^+ -depleted solution (NH_4^+ depletion). A. Pn (net photosynthesis rates measured at growth ambient CO_2 concentration and saturated light intensity); B. gs (stomatal conductance); C. Ci (intercellular CO_2 concentration); D. Ci/Ca (ratio of intercellular to air CO_2 concentration); E. Trmmol (transpiration rate); F. WUE (water use efficiency). Different letters indicate significant differences ($P < 0.05$) among treatments according to one-way ANOVA. Values are means \pm SD of four replicates; the experiments were repeated three times

the decline of gs. SE [CO_2] treatment greatly reduced gs (Fig. 2B). The intercellular CO_2 concentration (Ci), which was measured at each of the CO_2 concentrations tested, was lower after the SE than after the LE treatment, and NH_4^+ depletion did not influence this response (Fig. 2C). The ratio of intercellular CO_2 concentration to air CO_2 concentration (Ci/Ca) was not influenced by SE and NH_4^+ depletion; however, plants under LE treatment maintained a higher Ci/Ca than plants subjected to SE [CO_2] (Fig. 2D). Trends of changes of transpiration rate (Trmmol) were the same as those for gs (Fig. 2E). SE [CO_2]-treated seedlings had the highest WUE, which was independent of the type of nitrogen supply, and there was no significant difference in WUE between LA- and LE-treated plants fertilised with NH_4^+ -depleted solution (Fig. 2F).

The preferences of plants for different forms of inorganic N vary among species, and may be related to environmental factors (Peuke and Tischner, 1991; Bledsoe and Zasoski, 1983). Wheat and tea can grow in media in which NH_4^+ is the sole inorganic N

source (Bloom, 2009; Yang *et al.*, 2013), whereas *Juglans sigillata* grew poorly in such culture media owing to cell membrane damage caused by excessive accumulation of NH_4^+ (Fan *et al.*, 2013). The mechanism of CO_2 acclimation might be that E [CO_2] decreases the rate of photorespiration and inhibits the assimilation of NO_3^- into NH_4^+ . In wheat and *Arabidopsis* plants, the phenomenon of CO_2 acclimation arise due to the inhibition of N assimilation upon long-term treatment with NO_3^- (Bloom *et al.*, 2010). Interestingly, for *E. adenophorum*, E [CO_2] did not induce photosynthesis acclimation when provided with non-depleted Hoagland solutions. The acclimation of *E. adenophorum* in terms of its photosynthesis did not occur in LE [CO_2]-treated plants in the absence of NH_4^+ . This differs from the findings for wheat and *Arabidopsis* (Bloom *et al.*, 2010). Short-term [CO_2] elevation can greatly promote stomatal closure in *E. adenophorum* and the subsequent decline of Ci, which might explain photosynthesis-related acclimation after SE [CO_2] and NH_4^+ -depleted treatment. The ability of NH_4^+ depletion to inhi-

bit the promotion of photosynthesis by SE [CO₂] might also be attributable to the inhibition of NO₃⁻ assimilation, as reported for wheat and *Arabidopsis* (Bloom *et al.*, 2010). The considerable plasticity of *E. adenophorum* might have enabled it to adapt to the conditions of NH₄⁺ depletion by maintaining a high rate of photosynthesis.

Plants can maintain relatively constant Ci/Ca values by adjusting stomatal anatomy and chloroplast biochemistry (Frans *et al.*, 2013). NH₄⁺ depletion has no influence on the Ci/Ca ratio. The value of Ci/Ca in SE [CO₂]-treated plants was similar to that of LA [CO₂]-treated plants; however, plants subjected to long-term [CO₂] elevation maintained a higher Ci/Ca value. This indicated that only long-term (40 days) E [CO₂] adaptation can enable *E. adenophorum* to adjust its stomata and chloroplasts in order to adapt to E [CO₂]. The lower stomatal conductance of plants after NH₄⁺ depletion observed for the other nitrogen forms studied might be accounted for by the ability of NH₄⁺ to promote high mesophyll conductance (Guo *et al.*, 2002; Raab and Terry, 1994). CO₂ enrichment might promote WUE by decreasing both stomatal conductance and the rate of leaf transpiration (Conley *et al.*, 2001; Wullschlger, 2002). Therefore, invasive species with high-

er WUE induced by high CO₂ concentration grew faster than native species in water-limited ecosystems (Blumenthal *et al.*, 2013). However, CO₂ enrichment promoted WUE of *E. adenophorum* only under SE [CO₂], mainly as a consequence of the marked decrease of gs after short-term E [CO₂] exposure. However, LE [CO₂]-treated plants might have adapted to the conditions of CO₂ enrichment; they could maintain high levels of Pn and gs, which possibly contributed to the relatively low level of WUE.

2.3 Interactions of elevated CO₂ concentration and nitrogen source on A/Ci response curves of *E. adenophorum*

A/Ci response curves were used to detect photosynthetic acclimation to CO₂ enrichment in individual *E. adenophorum* leaves (Fig. 3). For plants grown in NH₄⁺-depleted Hoagland solution, the A/Ci response curves under LE [CO₂] were no different from those of plants under LA [CO₂] treatment. The initial slope of the A/Ci response curve of plants under SE [CO₂] treatment was 0.284 ± 0.0056 , which was 42% lower ($P < 0.05$) than that of leaves under long-term CO₂ treatment (including LA and LE [CO₂] treatments). There were no significant differences among the three CO₂ treatments in the upper portion of the A/Ci response curves. For plants

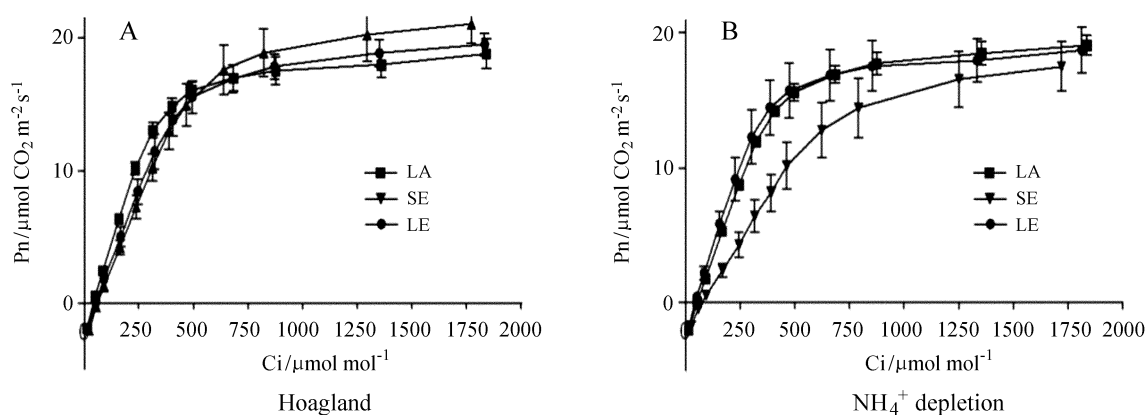


Fig. 3 Differences in A-Ci curves determined for *E. adenophorum* grown under conditions of long-term ambient CO₂ concentration (LA), short-term elevated CO₂ concentration (SE) and long-term elevated CO₂ concentration (LE) when fertilised with Hoagland solution (Hoagland) and NH₄⁺-depleted solution (NH₄⁺ depletion). A. Light-saturated A-Ci curves of *E. adenophorum* under non-depleted Hoagland solutions; B. Light-saturated A-Ci curves of *E. adenophorum* under NH₄⁺-depleted Hoagland solutions. Values are means \pm SD of three replicates; the experiments were repeated three times

grown in non-depleted Hoagland solution, there was almost complete overlap in the A/C_i response curves for the three CO_2 treatments.

The initial slope of the A/C_i response curve is determined by the total ribulose-1, 5-bisphosphate carboxylase oxygenase (Rubisco) activity (Sharkey, 2012; Voncaemmerer and Farquhar, 1981). Given the effect of Rubisco activity on the initial slope and the decreased initial Rubisco activity in wheat flag leaves under CO_2 enrichment (Sicher and Bunce, 1997) suggest that the fact that NH_4^+ depletion decreased the initial slope of the A/C_i response curve of plants under SE $[\text{CO}_2]$ treatment might have resulted from the decrease of Rubisco activity. Rubisco activity is regulated in order to maintain a balance with the capacity in RuBP regeneration, and the available products of photo-energy (ATP, NADPH) could determine the regeneration of RuBP (Sage *et al.*, 1988), and the photo-energy cost for ammonium supply is 145% less than for nitrate supply (Raven, 1985). Assimilation of NO_3^- in the leaf led to consumption of a substantial proportion of products of the electron transport chain of photosynthesis (Bloom *et al.*, 1989). Thus, some plants have higher rates of both CO_2 assimilation and RuBP regeneration per leaf area when fertilised with NH_4^+ rather than NO_3^- (Florian *et al.*, 2013). Given the higher rates of photosynthesis supported by NH_4^+ relative to NO_3^- , the inhibition of the increase in photosynthesis induced by SE $[\text{CO}_2]$ and the decrease of initial slope of the A/C_i response curve in *E. aden-*

ophorum fertilised with NO_3^- as the only N source suggest that the assimilation of NO_3^- alone might lead to the consumption of more products of photo-energy; the decreased available products of photo-energy used for photosynthesis might eventually inhibit the activity of Rubisco and thus affect the capacity for photosynthesis.

2.4 Effects of elevated $[\text{CO}_2]$ on photosynthetic pigment content

Photosynthetic pigment contents were determined to estimate potential changes in the photosynthetic capacity of *E. adenophorum* (Table 1). With non-depleted Hoagland solutions, there were no significant differences in the levels of chlorophyll and carotenoids among the three CO_2 treatments. In plants under NH_4^+ -depleted Hoagland solutions, the contents of chlorophyll were lower than for those grown in non-depleted Hoagland solutions under LA and LE $[\text{CO}_2]$. The plants grown under SE $[\text{CO}_2]$ exhibited higher levels of chlorophyll *a*, chlorophyll *b* and carotenoids than those grown in ambient atmospheric CO_2 conditions with NH_4^+ -depleted Hoagland solution. The increases in the levels of chlorophyll *a* and chlorophyll *b*, total chlorophyll and carotenoid levels in plant leaves were 31.8%, 37.0%, 25.0% and 30.4%, respectively. However, following the LE $[\text{CO}_2]$ treatment, the levels of chlorophyll *a*, *b*, total chlorophyll and carotenoids of plants decreased and were similar to those of plants grown under ambient $[\text{CO}_2]$ with NH_4^+ -depleted Hoagland solution. Rong *et al.* (2010) asserted that NH_4^+ can

Table 1 Difference in photosynthetic pigment content of *E. adenophorum* leaves under conditions of long-term ambient CO_2 concentration (LA), short-term elevated CO_2 concentration (SE) and long-term elevated CO_2 concentration (LE) when fertilised with Hoagland solution (Hoagland) and NH_4^+ -depleted solution (NH_4^+ depletion). Different letters indicate significant differences ($P < 0.05$) among treatments according to one-way ANOVA. Values are means \pm SD of four replicates; the experiments were repeated three times

	Hoagland			NH_4^+ depletion		
	LA	SE	LE	LA	SE	LE
Chlorophyll <i>a</i> ($\text{mg} \cdot \text{g}^{-1}$ DW)	11.59 \pm 1.53 ^{ab}	13.30 \pm 0.97 ^a	12.09 \pm 2.32 ^{ab}	9.33 \pm 1.37 ^c	12.30 \pm 0.68 ^{ab}	8.06 \pm 0.92 ^c
Chlorophyll <i>b</i> ($\text{mg} \cdot \text{g}^{-1}$ DW)	4.51 \pm 0.73 ^{ab}	5.28 \pm 1.02 ^a	4.41 \pm 0.81 ^{ab}	3.51 \pm 0.72 ^{bc}	4.81 \pm 0.18 ^a	3.06 \pm 0.34 ^c
Chlorophyll <i>a+b</i> ($\text{mg} \cdot \text{g}^{-1}$ DW)	16.10 \pm 2.23 ^a	18.59 \pm 1.97 ^a	16.50 \pm 3.09 ^a	12.84 \pm 2.09 ^b	17.11 \pm 0.85 ^a	11.11 \pm 1.23 ^b
Carotenoid ($\text{mg} \cdot \text{g}^{-1}$ DW)	2.47 \pm 0.36 ^{ab}	2.86 \pm 0.18 ^a	2.67 \pm 0.53 ^a	2.04 \pm 0.34 ^b	2.66 \pm 0.17 ^a	2.01 \pm 0.30 ^b

increase rates of chlorophyll synthesis in plants by stimulating the accumulation of the precursors of chlorophyll, such as glutamate and 2-oxoglutaric acid. NH₄⁺ depletion might deduce levels of photosynthetic pigments in LA- and LE [CO₂]-treated plants. However, short-term E [CO₂] could compensate for the reduced pigment synthesis observed in plants fertilised with NH₄⁺-depleted Hoagland solution. This increase of photosynthetic pigments also suggests that *E. adenophorum* with NH₄⁺-depleted Hoagland solution needed more photo-energy to complete NO₃⁻ assimilation and promote photosynthesis when grown under SE [CO₂] than when grown with non-depleted Hoagland solution.

2.5 Effects of elevated [CO₂] on plant NO₃⁻ status

The content of inorganic NO₃⁻ in *E. adenophorum* grown in non-depleted Hoagland solutions was not affected by the various CO₂ treatments. NH₄⁺ depletion greatly reduced the level of inorganic NO₃⁻ of LA- and LE [CO₂]-treated plants, especially in the latter group. However, the level of inorganic NO₃⁻ for SE [CO₂] under NH₄⁺-depleted conditions remained high, which is the same as the case of growing with non-depleted Hoagland solutions (Fig. 4). E [CO₂] might not influence the absorption and assimilation of NO₃⁻ with solutions that contain NH₄⁺. However, compared with short-term E [CO₂] treatment, NH₄⁺ depletion induced a marked decline in the NO₃⁻ content in LE [CO₂]-treated plants. A higher exogenous NO₃⁻ concentration could promote the activity of nitrate reductase in maize leaves (Wang *et al.*, 2009), whereas a high concentration of NH₄⁺ decreased nitrate reductase activity (Clausen and Lenz, 1999). Hoagland solutions depleted of NH₄⁺ had relatively high levels of NO₃⁻ compared with non-depleted Hoagland solutions. The high concentration of NO₃⁻ might increase nitrate reductase activity in *E. adenophorum* and reduce the residual concentration of NO₃⁻ in leaves (Fig. 4). Whereas SE [CO₂] treatment might inhibit the promotion of

nitrate reductase activity induced by NH₄⁺ depletion, and LE [CO₂] treatment eliminates this inhibition in *E. adenophorum*.

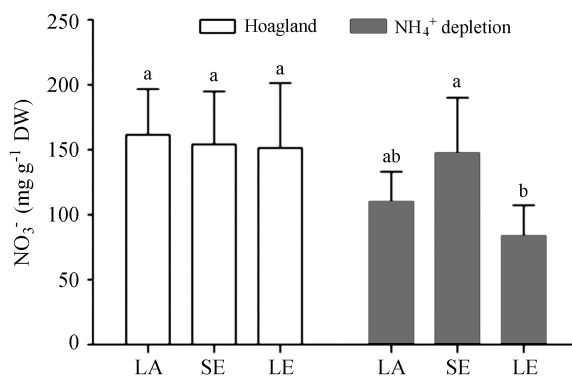


Fig. 4 Differences in NO₃⁻ content of *E. adenophorum* leaves under conditions of long-term ambient CO₂ concentration (LA), short-term elevated CO₂ concentration (SE) and long-term elevated CO₂ concentration (LE) when plants were fertilised with Hoagland solution (Hoagland) and NH₄⁺-depleted solution (NH₄⁺ depletion). Different letters indicate significant differences ($P < 0.05$) among treatments according to one-way ANOVA. Values are means \pm SD of four replicates; the experiments were repeated three times

3 Conclusion

This study of the invasive plant *E. adenophorum* investigated the effect of various treatments that combined an elevated [CO₂] with different forms of inorganic N. E [CO₂] promoted the photosynthesis of *E. adenophorum*, regardless of the duration of treatment with non-depleted Hoagland solution. Whereas NH₄⁺ depletion could inhibit the promotion of *E. adenophorum* photosynthesis by short-term E [CO₂]. The apparent ability of NO₃⁻ at LE [CO₂] to promote biomass accumulation and photosynthesis without a decrease in chlorophyll indicates that *E. adenophorum* might not acclimate to long-term exposure to E [CO₂]. Thus, considering some plants fertilised with NO₃⁻ acclimating to LE [CO₂], *E. adenophorum* might be more competitive in areas where the soils are relatively poor in NH₄⁺ as levels of atmospheric CO₂ continue to rise.

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